Dietary glucosinolate intake and risk of prostate cancer in the EPIC-Heidelberg cohort study

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Glucosinolates (GLS) are secondary plant metabolites occurring in cruciferous vegetables. Their biologically active break-down products show cancer preventive properties in animal and cell studies. So far, epidemiologic studies, using consumption of cruciferous vegetables as proxy for GLS intake, yielded inconsistent results. Here, we evaluated the association between dietary intake of GLS in comparison with consumption data of GLS-containing foods and the risk of prostate cancer. The study population comprised 11,405 male participants of the prospective EPIC-Heidelberg cohort study. During a mean follow-up time of 9.4 years, 328 incident cases of prostate cancer occurred. At recruitment, habitual food consumption was assessed by a validated food frequency questionnaire, and intake of individual GLS was estimated by means of a newly compiled database on food content of GLS. Adjusted hazard ratios (HR) for prostate cancer were calculated using the Cox proportional hazard model. Median daily intake of total GLS was 7.9 mg/day (interquartile range 5.1–11.9 mg/day). The risk of prostate cancer decreased significantly over quartiles of total GLS intake (multivariate HR [4th *vs.* 1st quartile] 0.68, 95% CI 0.48–0.97, p_{trend} 0.03). Associations with GLS-containing food intake were weaker. Among GLS subgroups, aliphatic GLS showed the strongest inverse association with cancer risk. Analyses stratified by tumor stage and grade gave hint to inverse associations for localized and low-grade cancers. This study shows an inverse association between dietary intake of GLS and the risk of prostate cancer. Because this is the first prospective study using individual GLS intake data, confirmation in other studies is warranted.

Glucosinolates (GLS) are plant metabolites occurring predominantly in cruciferous vegetables.[1] According to the chemical structure of their side chain 3 main groups can be distinguished: aliphatic (with alkyl or alkenyl group), aromatic (with benzyl group) and heterocyclic (with indolyl group) GLS. On cell rupture, GLS are cleaved by the plant enzyme myrosinase, and depending on the structure and reaction conditions, various biologically active components can be formed.[2] Isothiocyanates—derived from aliphatic and aromatic GLS (at neutral pH)—and indoles—derived from indolyl-GLS—are currently the break-down products of most scientific interest because of their cancer preventive properties observed in cell and animal studies.[3]

Different mechanisms of anticarcinogenic activity are proposed for these break-down products such as the induction of antioxidant and detoxification genes. Furthermore, the inhibition of proinflammatory reactions, cytochrome P450 enzyme activity and histone deacetylase are under discussion as well as the activation of cell cycle arrest and apoptosis.[4] In prostate cancer cells lines it was shown, that sulforaphane, the hydrolysis product of glucoraphanine as the major aliphatic GLS in broccoli, was a potent inducer of the detoxification enzyme quinone oxidoreductase.[5] Similarly, indole-3-carbinol derived from indolyl GLS inhibited the growth of PC-3 cells (prostate cancer cells) and regulated the expression of apoptosis-related genes.[6]

Anticarcinogenic effects in human prostate cancer still have to be established. So far, case–control studies[7–16] and prospective cohort studies[17–23] observed inconsistent results. All these studies used the intake of cruciferous vegetables as a proxy for GLS intake. Additionally in some studies, the intake of only 1 type of cruciferous vegetables or a small group of cruciferous vegetables was considered,[10,11,14,18,19,23] *i.e.*, ignoring other dietary sources of GLS. Because types and amount of GLS in different cruciferous vegetables vary considerably, direct quantification of individual GLS intake on the basis of a full list of GLS-containing foods would provide an important improvement in exposure measurement. Recently, a database on the content of individual GLS in food was established covering 18 different GLS-containing foods commonly consumed by humans.[24] Thus, this database allows for intake calculation of individual GLS, GLS subgroups and total GLS.

It was the aim of this study to evaluate the association between dietary intake of GLS and GLS subgroups and the risk of prostate cancer in the EPIC-Heidelberg cohort study. For comparison, a specific food group of GLS-containing foods was created, covering 18 different vegetables and condiments, to evaluate associations with prostate cancer risk.

Material and methods

Study population

The EPIC-Heidelberg cohort is part of the European Prospective Investigation into Cancer and Nutrition, a multicentric prospective cohort study assessing the relationship between dietary, lifestyle and metabolic factors and the risk of chronic diseases, especially cancer.[25] From 1994 to 1998, a random sample of the general population of Heidelberg, Germany, and surrounding communities was invited for participation in the EPIC-Heidelberg cohort study. A total of 11,928 men aged 40–64 years and 13,612 women aged 35–64 years were recruited, which comprises 38% of those approached. The cohort deviates from the underlying population in terms of more favorable socioeconomic and health indicators.[26] At baseline, dietary, lifestyle, medical and socioeconomic data were collected and anthropometric measures were assessed by trained personnel. All participants gave written informed consent. The study was approved by the ethics committee of the Heidelberg Medical School. The cohort is followed up by mailed questionnaires in intervals of about 3 years to assess information on health status.[27] In all of the 3 completed follow-up rounds, participation rates were above 90% ensuring high cohort-internal validity.

After exclusion of participants with prevalent cancer other than nonmelanoma skin cancer at baseline ($n = 375$), with no follow-up data available ($n = 33$) and those in the top and bottom 0.5 percentile of the ratio of energy intake to basal metabolic rate ($n = 115$), the analytic cohort comprised 11,405 men.

Data collection and dietary intake calculation

Habitual diet during the previous year was assessed through a validated self-administered semiquantitative food frequency

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questionnaire. Participants were asked to fill in portion size and consumption frequency of 145 food items.[28,29] The dietary information was used to determine average daily food intake for each participant; by linking food consumption data to the German Food Code and Nutrient Data Base BLS II.3 (BgVV, Berlin, Germany), the intake of individual nutrients were calculated. Because the BLS does not capture GLS contents of foods, dietary data were linked to a database on individual and total GLS content of food especially compiled for this purpose.[24] In brief, 18 GLS-containing vegetables and condiments (broccoli, cauliflower, Brussels sprouts, white, red, Savoy and Chinese cabbage, sauerkraut, kale, kohlrabi, turnip, swede, radish, horseradish, mustard, capers, water and garden cress) commonly consumed in Germany were identified. A literature search was conducted to assemble studies analyzing the contents of individual GLS via high-performance liquid chromatography or gas chromatography in these foods to calculate median contents of individual GLS per 100 g fresh weight. Thus, it was possible to calculate the average daily intake of 26 individual GLS, GLS subgroups (aliphatic, aromatic, indolyl) and total GLS for each participant. Furthermore, a special food group was formed by summing up daily consumption (in grams) of the aforementioned GLS-containing foods for each participant. When these foods were part of a mixed dish, they were also considered in the GLS-containing food group.

At recruitment, nondietary data were assessed by self-administered questionnaire and personal interview. Questions covered occupational situation, education, socioeconomic status, physical activity at work and during leisure time, lifetime consumption of alcohol and tobacco and medical history. Furthermore, anthropometric measures were taken by trained personnel at the study centre following standardized procedures.[30]

Follow-up questionnaires are mailed to each participant in regular intervals to collect data on newly diagnosed diseases. Each self-reported case of prostate cancer was verified by the study physician based on medical records. Additionally, death certificates are examined for prostate cancer as underlying cause of death. For this analysis, only incident and verified cases of prostate cancer (C61, C63.8 and C63.9; International Classification of Diseases for Oncology, 2nd edition) were included. Information on tumor stage (tumor (T)-nodal (N)-metastasis (M) categories) and grade (Gleason histologic grade) was collected from medical (pathology) records by trained physicians. Tumor stage was categorized as advanced (T3 or T4, N1 or N2, M1 or some combination of these), localized (T0 or T1 or T2 and N0 or NX and M0) or unknown. Tumor grade was classified as high grade (Gleason sum $\geq$ 7), low-grade (Gleason sum $<$ 7) or unknown. During the second, third and fourth follow-up, participants were asked for occurrence of cancer in first-grade relative and if they had undergone prostate-specific antigen (PSA) screening.

Statistical analysis

Participants were divided by quartiles of intake of total GLS, GLS subgroups or GLS-containing food consumption. Baseline characteristics of the study population over intake quartiles are given as percentages for categorical variables and as arithmetic mean $\pm$ standard deviation or median and interquartile range for continuous variables.

The person-time each participant contributed was calculated as difference between the date of entry into the study until the date of diagnosis of prostate cancer, the date of death or the date of last known contact, whichever came first. Participants diagnosed with other cancers (excluding nonmelanoma skin cancer) were censored at the date of diagnosis.

The Cox proportional hazard model was used to analyze the association between the intake of GLS, GLS subgroups or GLS-containing foods and prostate cancer. Hazard Ratios (HR) and 95% confidence intervals (CI) were calculated over intake quartiles. Tests for linear trend were performed by assigning the median of the respective intake quartile to each participant and using it as a continuous variable. Analyses were repeated after excluding person-time and incident cases of the first 2 years of follow-up and also in the subcohort of study subjects (cases and noncases) who had undergone PSA screening. Furthermore, analyses were performed by tumor stage (advanced and localized) and tumor grade (high grade and low grade). We tested for heterogeneity by outcome strata using the data augmentation method by Lunn and McNeil.[31] For the quartile model, the p value of the likelihood ratio test statistics comparing the model with and without interaction terms is presented. All models were stratified by age (1 year categories). Multivariate models were adjusted for intake of vegetables excluding GLS-containing vegetables and tomatoes (quartiles), intake of tomatoes and tomato products (quartiles), intake of milk and milk products (quartiles), energy intake from fat (quartiles), nonalcohol–nonfat energy intake (quartiles), alcohol intake ($<$ 5 g/day, 5–15 g/day, 15–30 g/day, $\geq$30 g/day), educational attainment (none or primary school, technical school, secondary school, university degree), smoking status (never, former, current), total physical activity (inactive, moderately inactive, moderately active, active), body mass index (kg/m^2), waist-to-hip ratio ($<$1, $\geq$1) and family history of prostate cancer (yes, no). All statistical analyses were performed with SAS 9.1 (SAS Institute, Cary, NC).

Results

During an average of 9.4 years of follow-up, 328 of the 11,405 men were diagnosed with prostate cancer; 37 got a diagnosis within the first 2 years of follow-up. Using data on tumor stage, 77 were classified as advanced and 188 as localized cases (n = 63 missing). Data on Gleason sum score were available for 247 of the cancer cases with 104 being categorized as high-grade cancer (Gleason sum $\geq$ 7) and 143 as low-grade (Gleason sum $<$ 7). Fifty-two percent of the men in the cohort had undergone PSA screening.

At baseline, the median dietary intake (interquartile range) of total GLS was 7.9 (5.1–11.9) mg/day in the entire male cohort. The main proportion was attributed to the intake of aliphatic GLS with a median intake of 4.2 (2.6–6.5) mg/day. Intake of indolyl and aromatic GLS was distinctly lower with 2.8 (1.7–4.4) and 0.7 (0.4–1.1) mg/day, respectively. The intake of aliphatic and indolyl GLS were highly correlated with a Pearson correlation coefficient of 0.91. The median intake of GLS-containing foods was 15.8 (10.0–24.3) g/day.

Baseline characteristics of the study population over quartiles of GLS and GLS-containing food intake are given in Table I. Age at baseline as well as intake of energy, fat, vegetables, tomato and tomato products and milk products tended to increase with increasing quartile of intake. Participants in the higher intake quartiles were more likely to have had PSA screening and to be engaged in physical activity, whereas they were less likely to be current smokers. Body mass index, waist-to-hip ratio, family history of prostate cancer, educational attainment, alcohol and milk intake were nearly equally distributed over quartiles. Crosstabulation of quartiles of GLS intake and GLS-containing food consumption showed that 82% were categorized into the same quartiles and 17.5% into adjacent quartiles. There was no tendency for a systematic misclassification (data not shown).

Associations between total GLS intake and prostate cancer risk are shown in Table II. The hazard ratio for prostate cancer decreased constantly over quartiles of GLS intake in multivariately adjusted models with a significantly reduced HR of 0.68 (CI: 0.48–0.97) for the 4th *vs*. the 1st intake quartile (p_{trend} 0.03). After excluding the first 2 years of follow-up or limiting the study population to those who had undergone at least 1 PSA screening yielded again results similar to those obtained in the full cohort. Analyses stratified by tumor stage or grade revealed significantly inverse associations of GLS intake for localized and marginally significant associations for low-grade prostate cancer but not for advanced or high-grade cases. The test for differences between

TABLE I – BASELINE CHARACTERISTICS OF MEN OF THE EPIC-HEIDELBERG COHORT OVER QUARTILES OF TOTAL GLUCOSINOLATE INTAKE OR GLUCOSINOLATE-CONTAINING FOOD CONSUMPTION

	Total glucosinolate intake (mg/day)				Glucosinolate-containing food consumption (g/day)			
	1st quartile (<5.1)	2nd quartile (5.1 to <7.9)	3rd quartile (7.9 to <11.9)	4th quartile (≥11.9)	1st quartile (<10.0)	2nd quartile (10.0 to <15.8)	3rd quartile (15.8 to <24.3)	4th quartile (≥24.3)
N total	2851	2851	2852	2851	2851	2851	2852	2851
Mean ± standard deviation								
Age at baseline (year)	50.5 ± 7.0	51.8 ± 7.1	52.5 ± 7.2	53.0 ± 7.0	50.5 ± 7.0	51.8 ± 7.1	52.5 ± 7.2	53.0 ± 7.0
Body mass index (kg/m^2)	26.9 ± 3.6	27.0 ± 3.6	27.0 ± 3.6	26.9 ± 3.8	26.9 ± 3.6	27.0 ± 3.6	27.0 ± 3.6	27.0 ± 3.8
Waist-to-hip ratio	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
Family history of prostate cancer (%)	3.9	3.4	3.8	2.8	3.9	3.3	3.6	3.1
Participation in PSA screening (%)	49.9	51.7	53.5	53.0	49.0	52.6	53.7	52.8
Smoking status (%)								
Never	28.9	29.0	30.6	32.2	28.6	28.7	30.9	32.5
Former	44.8	46.2	47.4	45.7	45.6	46.4	46.8	45.2
Current	26.3	24.8	22.0	22.1	25.8	24.9	22.3	22.2
Educational attainment (%)								
None/primary school	31.5	30.7	31.0	31.7	30.4	31.6	30.5	32.3
Technical school	27.0	28.3	26.8	25.2	26.9	28.0	27.1	25.3
Secondary school	5.9	5.3	5.2	5.9	6.2	5.2	5.1	5.7
University	35.6	35.7	37.0	37.3	36.4	35.2	37.2	36.8
Total physical activity (%)								
Inactive	36.7	33.8	33.6	33.4	36.2	34.3	33.2	33.7
Moderately inactive	31.6	32.8	30.2	30.9	31.9	32.3	30.6	30.8
Moderately active	25.5	26.8	29.2	27.6	25.7	26.7	29.7	27.2
Active	6.2	6.6	7.0	8.1	6.2	6.8	6.5	8.3
Nutrient intake								
Total energy (kcal/day)	2044 ± 649	2166 ± 658	2245 ± 690	2368 ± 731	2063 ± 658	2162 ± 663	2251 ± 688	2348 ± 729
Total fat (g/day)	76.1 ± 30.5	81.7 ± 30.0	85.0 ± 32.0	91.6 ± 35.8	77.2 ± 30.7	81.5 ± 30.5	85.1 ± 32.2	90.6 ± 35.4
Alcohol (g/day)	25.2 ± 27.5	25.9 ± 26.3	26.5 ± 27.6	25.9 ± 27.2	25.5 ± 27.6	26.2 ± 27.5	26.0 ± 27.1	25.7 ± 26.5
Food intake (g/day, median (interquartile range))								
All vegetables	71.4 (53.0–92.1)	94.1 (76.6–114.3)	111.4 (91.7–137.5)	143.9 (114.0–184.3)	71.8 (53.2–93.4)	94.5 (76.7–115.5)	110.7 (91.0–135.5)	143.2 (113.0–182.9)
Selected vegetables[1]	48.4 (34.5–64.7)	64.5 (50.2–81.2)	75.7 (57.9–96.9)	90.2 (66.8–122.7)	49.0 (34.7–65.8)	64.8 (50.7–82.6)	74.6 (57.4–95.7)	89.0 (65.3–121.0)
Tomatoes, tomato products	18.5 (11.7–27.3)	20.7 (13.7–29.2)	22.0 (14.5–31.3)	23.2 (15.3–33.8)	18.7 (11.9–27.9)	20.8 (13.6–29.6)	21.9 (14.6–31.0)	22.9 (15.0–33.3)
Milk	59.4 (20.0–151.2)	62.3 (22.7–150.6)	61.9 (21.1–150.0)	64.9 (22.7–155.6)	57.2 (19.7–151.3)	63.4 (22.1–150.7)	62.6 (21.8–151.6)	64.8 (22.8–154.3)
Milk products	72.2 (39.2–136.4)	81.9 (45.5–142.8)	86.5 (47.1–150.8)	91.1 (49.1–163.1)	73.1 (39.9–137.3)	82.7 (45.4–145.3)	85.5 (47.1–149.0)	90.7 (48.2–162.1)

[1]Selected vegetables: vegetables excluding glucosinolate-containing foods and tomatoes/tomato products.

TABLE II – HAZARD RATIO (HR) AND 95% CONFIDENCE INTERVAL (CI) FOR PROSTATE CANCER OVER QUARTILES OF TOTAL GLUCOSINOLATE INTAKE OR GLUCOSINOLATE-CONTAINING FOOD CONSUMPTION IN MEN OF THE EPIC-HEIDELBERG COHORT

	Total glucosinolate intake (mg/day)					Glucosinolate-containing food consumption (g/day)				
	1st quartile (<5.1)	2nd quartile (5.1 to <7.9)	3rd quartile (7.9 to <11.9)	4th quartile (≥11.9)	p_{trend}[2]	1st quartile (<10.0)	2nd quartile (10.0 to <15.8)	3rd quartile (15.8 to <24.3)	4th quartile (≥24.3)	p_{trend}
Total prostate cancer										
N cases	78	85	86	79		75	84	91	78	
Age-stratified HR (CI)	1.00	0.92 (0.67, 1.25)	0.85 (0.63, 1.16)	0.75 (0.55, 1.03)	0.07	1.00	0.95 (0.69, 1.30)	0.94 (0.69, 1.27)	0.77 (0.56, 1.07)	0.10
MV[1]-adjusted HR(CI)	1.00	0.88 (0.64, 1.21)	0.78 (0.56, 1.09)	0.68 (0.48, 0.97)	0.03	1.00	0.91 (0.66, 1.25)	0.87 (0.63, 1.21)	0.72 (0.51, 1.02)	0.05
Excluding first 2 years of follow-up										
N cases	70	70	80	71		66	71	85	69	
MV-adjusted HR(CI)	1.00	0.81 (0.57, 1.14)	0.82 (0.58, 1.16)	0.68 (0.47, 0.99)	0.07	1.00	0.88 (0.62, 1.24)	0.94 (0.66–1.32)	0.72 (0.50, 1.05)	0.09
Only participants with PSA screening										
N cases	74	75	78	70		70	75	84	68	
MV-adjusted HR(CI)	1.00	0.85 (0.61, 1.18)	0.74 (0.53, 1.05)	0.65 (0.45, 0.94)	0.02	1.00	0.84 (0.60, 1.18)	0.83 (0.59, 1.17)	0.65 (0.45, 0.94)	0.03
Advanced prostate cancer										
N cases	17	20	27	13		20	14	29	14	
MV-adjusted HR(CI)	1.00	1.05 (0.54, 2.04)	1.23 (0.64, 2.36)	0.61 (0.28, 1.36)	0.19	1.00	0.61 (0.30, 1.25)	1.14 (0.61, 2.10)	0.56 (0.26, 1.18)	0.26
Localized prostate cancer										
N cases	48	45	46	49		43	48	50	47	
MV-adjusted HR(CI)	1.00	0.71 (0.47, 1.09)	0.63 (0.41, 0.97)	0.63 (0.40, 0.98)	0.09	1.00	0.87 (0.57–1.33)	0.78 (0.51, 1.21)	0.70 (0.45, 1.11)	0.14
$p_{difference}$[3] between outcome strata					0.20					0.20
High-grade prostate cancer										
N cases	25	22	33	24		23	18	36	27	
MV-adjusted HR(CI)	1.00	0.78 (0.43, 1.40)	1.03 (0.59, 1.81)	0.74 (0.39, 1.39)	0.47	1.00	0.74 (0.39, 1.40)	1.33 (0.76, 2.33)	1.01 (0.54, 1.87)	0.69
Low-grade prostate cancer										
N cases	35	38	35	35		34	40	38	31	
MV-adjusted HR(CI)	1.00	0.81 (0.50, 1.30)	0.65 (0.39, 1.06)	0.60 (0.35, 1.01)	0.06	1.00	0.84 (0.52, 1.35)	0.70 (0.43, 1.15)	0.53 (0.31, 0.91)	0.02
$p_{difference}$ between outcome strata					0.59					0.18

[1]Multivariate model stratified by age (years) and adjusted for intake of other vegetables, tomatoes, tomato products, milk, milk products, alcohol, energy from fat, nonalcohol-nonfat energy, educational attainment, smoking status, total physical activity, body mass index, waist-to-hip ratio and family history of prostate cancer.

[2]p_{trend}: test for trend modeling median of the quartiles as continuous variable.

[3]$p_{difference}$: test for heterogeneity between outcome strata (advanced *vs.* localized stage prostate cancer, high-grade *vs.* low-grade prostate cancer).

outcome strata was not significant with respect to tumor stage ($p_{difference}$ 0.20) or tumor grade ($p_{difference}$ 0.59).

In comparison with these results, the association between intake of GLS-containing foods and prostate cancer showed similar tendencies albeit the strength of association was smaller and hazard ratios and trend tests did less often reach statistical significance (Table II).

Table III gives the hazard ratios for prostate cancer by intake quartiles of aliphatic and indolyl GLS. The intake of aliphatic GLS was inversely associated with the risk of prostate cancer in adjusted models (p_{trend} 0.04). After exclusion of the first 2 years of follow-up similar results were found. Likewise, the intake of aliphatic GLS showed a significantly inverse relationship with the risk of prostate cancer when only the subgroup of participants reporting PSA screening was analyzed [HR (4th *vs.* 1st quartile): 0.68, CI: 0.47–0.98, p_{trend} 0.03]. The inverse associations were again more pronounced for localized and low-grade cancers; however, the test for heterogeneity by outcome strata was not significant. Results for indolyl GLS showed similar tendencies as seen for aliphatic GLS; however, the associations were less strong. The test for differences of indolyl GLS intake quartiles for high- and low-grade cancer subtype was significant ($p_{difference} < 0.01$). Intake of aromatic GLS was not associated with prostate cancer risk (data not shown).

Discussion

We observed a statistically significant inverse association between intake of GLS, especially aliphatic GLS, and the risk of prostate cancer. This association was more pronounced for localized or low-grade cancer cases. Using data on the consumption of GLS-containing foods, associations were in the same direction but less strong.

To our knowledge, there is no other epidemiological study examining the association between intake of GLS or GLS subgroups and prostate cancer to date. However, some studies evaluated the association of prostate cancer and consumption of cruciferous vegetables. Because GLS occur nearly exclusively in cruciferous vegetables, their consumption may serve as a proxy for GLS intake. So far, these studies provide inconsistent results. Eight case–control studies established inverse associations between intake of cruciferous vegetables and prostate cancer risk,[7–14] whereas 2 others[15,16] could not find any relationship. A different picture arises from cohort studies, although a majority of cohort studies reported no relationship at all,[17–20] 2 studies gave indication for an inverse association,[21,22] and 1 showed borderline significant inverse associations.[23] Giovannucci *et al.*[21] found a weak and nonsignificant inverse association between cruciferous vegetable intake and prostate cancer in the Health Professional follow-up study. However, restricting the analysis to participants who had had a PSA test led to a significantly reduced risk of prostate cancer for the highest *vs.* lowest intake quintile (HR 0.77, CI 0.64–0.93, p_{trend} 0.03). Furthermore, the protective effect was found predominantly in younger men (diagnosed with cancer before age of 65 years) and in those with organ-confined (localized) prostate cancers. Kirsh *et al.*[22] evaluated the risk of prostate cancer in men of the screening arm of the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, all of which had undergone at least 1 PSA test. They found a nonsignificant association between cruciferous vegetable intake and total prostate cancer (HR (5th *vs.* 1st intake quintile) 0.85, CI 0.71–1.02, p_{trend} 0.08). However, advanced cancer (Stages III and IV) risk was significantly reduced for the 5th *vs.* the 1st intake quintile. Furthermore, they observed significantly inverse associations when focusing on the intake of single vegetables (broccoli or cauliflower) and advanced cancer. Schuurman *et al.*[23] reported nonsignificant inverse rate ratios for quintiles of increasing Brassica consumption (RR (5th *vs.* 1st intake quintile) 0.81, CI 0.59–1.12) with a nearly significant p for trend (0.06) in multivariate adjusted analyses of the Netherlands Cohort Study. Thus, with respect to total prostate cancer, the earlier presented studies are in accordance with our analyses of GLS-containing food intake, where we found weak and often nonsignificant inverse associations. On the other hand, our analyses stratified by tumor stage support the findings of Giovannucci and colleagues.[21]

Comparison of study results is always difficult because of differences in study design and population, statistical analysis or exposure and end point measurement. Giovannucci *et al.*[21] and Kirsh *et al.*[22] described the intake of cruciferous vegetables in their populations as servings per week, and other cohort studies used fewer vegetables to compute their GLS-containing food consumption data than we did. For the Spanish male EPIC cohort, a median intake of cruciferous vegetables of 4.1 g/day was reported based on data obtained by diet history method,[32] which is considerably less than the median intake in our study (15.8 g/day). However, this may be largely explained by a higher consumption of cabbages in Germany because of national consumption preferences.[33] Interestingly, a distinct difference between prospective studies that report an inverse association and those finding no relationship is the number of vegetables considered for the calculation of the cruciferous vegetables food group. Both, Giovannucci *et al.*[21] and Kirsh *et al.*,[22] used 9 different vegetables and condiments to build a cruciferous vegetable food group. In contrast, prospective studies finding no association based their calculations on less GLS-containing vegetables (maximum 5 items). In our study, the GLS-containing food group was also calculated as precisely as possible, and, finally, 18 vegetables and condiments were included. According to the results of earlier analyses in our cohort,[24] broccoli, Brussels sprouts and cauliflower are the foods contributing most to the intake of total and indolyl GLS. Nevertheless, vegetables such as swede/turnip, mustard, horseradish, cress and capers had a share of 20% of total GLS intake. For aliphatic GLS intake, the main food sources were radish (22%), broccoli and Brussels spouts. The interesting point here is that radish is a food that is not considered in any of the other epidemiologic studies on cruciferous vegetables and prostate cancer risk. Further, we checked whether these 18 foods were part of mixed dishes and added them to the food group. In addition to our comprehensive GLS-containing food group, we also performed an analysis by simply using the single food frequency item on the consumption of cabbages, cauliflower, broccoli and kohlrabi as exposure variable. This variable ignored other GLS-containing vegetables such as radishes, turnip or mustard, and it does not include GLS-containing foods, which are part of mixed dishes. When using this variable, we found no association with the risk of prostate cancer, the adjusted HR (CI) being 1.04 (0.76–1.43), 0.85 (0.61–1.18) and 0.92 (0.67–1.27) for the 2nd, 3rd and 4th intake quartile, respectively (p_{trend} 0.49). This emphasizes the need for a thorough assessment of all relevant GLS-containing foods if a direct quantification of GLS intake is not possible.

The results of the analysis based on directly quantified GLS intake data showed a clear and significant inverse association with prostate cancer, which supports the hypothesis that GLS, or more precisely GLS break-down products, are responsible for the anticarcinogenic activity of cruciferous vegetables.[34] Furthermore, when we repeated the analysis mutually adjusting for GLS intake and GLS-containing food consumption, the inverse association with GLS intake remained, indicating that not other compounds in GLS-rich vegetables are responsible for the effect. Although the GLS intake is highly correlated with the intake of GLS-containing food, it is a more precise measure of the potential biologically active components and might therefore lead to less misclassification. This is easily explained by the fact that amount and composition of individual GLS vary substantially between different foods. For example, 100 g of broccoli provide a different amount of various GLS than 100 g of Brussels sprouts, which is not accounted for when simply adding up the amount of both vegetables. Furthermore, subanalyses revealed that especially GLS with an aliphatic side chain were inversely correlated with the risk of

TABLE III – HAZARD RATIO (HR) AND 95% CONFIDENCE INTERVAL (CI) FOR PROSTATE CANCER OVER INTAKE QUARTILES OF ALIPHATIC OR INDOLYL GLUCOSINOLATES IN MEN OF THE EPIC-HEIDELBERG COHORT

	Aliphatic glucosinolate intake (mg/day)						Indole glucosinolate intake (mg/day)					
	1st quartile (< 2.6)	2nd quartile (2.6 to <4.2)	3rd quartile (4.2 to <6.5)	4th quartile (≥ 6.5)	p_{trend}[2]		1st quartile (< 1.7)	2nd quartile (1.7 to <2.8)	3rd quartile (2.8 to <4.4)	4th quartile (≥ 4.4)	p_{trend}	
Total prostate cancer												
N cases	77	88	83	80			77	82	87	82		
Age-stratified HR(CI)	1.00	0.99 (0.73, 1.35)	0.84 (0.61, 1.15)	0.78 (0.57, 1.07)	0.07		1.00	0.92 (0.67, 1.26)	0.93 (0.68, 1.26)	0.82 (0.60, 1.12)	0.22	
MV[1]-adjusted HR(CI)	1.00	0.94 (0.69, 1.29)	0.79 (0.57, 1.09)	0.71 (0.50, 1.01)	0.04		1.00	0.89 (0.65, 1.23)	0.87 (0.63, 1.20)	0.77 (0.55, 1.08)	0.14	
Excluding first 2 years of follow-up												
N cases	69	73	78	71			68	68	82	73		
MV-adjusted HR(CI)	1.00	0.88 (0.63, 1.23)	0.83 (0.59, 1.18)	0.71 (0.49, 1.02)	0.07		1.00	0.83 (0.59, 1.18)	0.94 (0.67, 1.32)	0.77 (0.54, 1.10)	0.23	
Only participants with PSA screening												
N cases	72	79	75	71			71	74	79	73		
MV-adjusted HR(CI)	1.00	0.92 (0.66, 1.27)	0.76 (0.54, 1.08)	0.68 (0.47, 0.98)	0.03		1.00	0.87 (0.62, 1.22)	0.82 (0.58, 1.14)	0.73 (0.51, 1.04)	0.09	
Advanced prostate cancer												
N cases	17	22	23	15			17	17	26	17		
MV-adjusted HR(CI)	1.00	1.19 (0.62, 2.29)	1.10 (0.57, 2.14)	0.71 (0.33, 1.52)	0.24		1.00	0.92 (0.46, 1.83)	1.31 (0.69, 2.50)	0.87 (0.42, 1.79)	0.76	
Localized prostate cancer												
N cases	49	45	44	50			46	48	46	48		
MV-adjusted HR(CI)	1.00	0.71 (0.47, 1.07)	0.61 (0.40, 0.93)	0.64 (0.42, 0.99)	0.11		1.00	0.86 (0.57, 1.30)	0.73 (0.48, 1.12)	0.71 (0.46, 1.09)	0.13	
$p_{difference}$ between outcome strata[3]						0.38						0.57
High-grade prostate cancer												
N cases	25	22	31	26			23	14	37	30		
MV-adjusted HR(CI)	1.00	0.76 (0.42, 1.38)	1.00 (0.57, 1.75)	0.80 (0.43, 1.48)	0.66		1.00	0.58 (0.30, 1.15)	1.46 (0.84, 2.53)	1.15 (0.64, 2.08)	0.26	
Low-grade prostate cancer												
N cases	35	41	33	34			35	45	32	31		
MV-adjusted HR(CI)	1.00	0.89 (0.56, 1.42)	0.63 (0.38, 1.04)	0.59 (0.35, 0.99)	0.03		1.00	1.00 (0.63, 1.57)	0.63 (0.38, 1.05)	0.55 (0.33, 0.93)	0.01	
$p_{difference}$ between outcome strata						0.49						<0.01

[1]Multivariate model stratified by age (years) and adjusted for intake of other vegetables, tomatoes, tomato products, milk, milk products, alcohol, energy from fat, nonalcohol-nonfat energy, educational attainment, smoking status, total physical activity, body mass index, waist-to-hip ratio and family history of prostate cancer.

[2]p_{trend}: test for trend modeling median of the quartiles as continuous variable.

[3]$p_{difference}$: test for heterogeneity between outcome strata (advanced *vs*. localized stage prostate cancer, high-grade *vs*. low-grade prostate cancer).

prostate cancer. Here, the intake of aliphatic and indolyl GLS is highly correlated because each cruciferous vegetable contains members of both subgroups; thus, mutually adjusted analyses are not reasonable.

Further support for the results presented here evolves from cell and animal studies on the anticarcinogenic properties of GLS break-down products.[3] Besides other proposed mechanisms, isothiocyanates, the hydrolysis products of aliphatic and aromatic GLS, induce detoxification enzymes, such as glutathione *S*-transferase or NAD(P)H:quinone oxidoreductase 1,[4] which are important for the excretion of toxic compounds from the body.[34] Derivatives of indolyl GLS, such as indolo[3,2-b]carbazole or 3,3′-diindolylmethane, induce the expression of CYP1A1.[4] Such Phase I enzymes activate toxic compounds, but this process also makes them accessible for detoxification by Phase II enzymes. The complete lack of association between intake of aromatic GLS and prostate cancer in our analyses might be due to exposure misclassification. The group of aromatic GLS consists of 4 compounds, which are found predominantly in vegetables and condiments that are hard to quantify by food frequency questionnaires, *e.g.*, mustard or cress.

Our results stratified by tumor stage and grade indicated significantly inverse associations of GLS intake with localized and low-grade prostate cancer. However, tests for heterogeneity over outcome strata showed no significant p values. It has to be taken into account that the number of cases in the advanced stage and high-grade strata was considerably small and thus statistical power was limited. Generally, our findings are in accordance with those of Giovannucci *et al.*[21] who consequently hypothesized that protective effects of cruciferous vegetables might act in early stages of tumor initiation or in the subgroup of less aggressive prostate cancer. In contrast, cell and animal studies suggest that isothiocyanates and indoles have the potential to act during later stages of cancer promotion and progression, because they impact on cell cycle arrest and apoptosis. However, it seems that for these chemopreventive mechanisms higher doses of GLS hydrolysis products are required, which might probably not be achieved in vivo.[4]

Also in our study, exposure misclassification for GLS intake cannot be ruled out. The level of GLS in foods is influenced by various factors such as cultivar type, growing conditions or harvest time,[35] for which we cannot fully control here. Similarly, storage time, processing conditions and preparation methods affect GLS contents in food.[36] Another important aspect is the limited knowledge about bioavailability and metabolism of GLS or their hydrolysis products in humans.[2] Thus, intake data can only give limited information about the actually available amount of compounds entering the circulation and probably revealing biological effects. It is also described that break-down products other than isothiocyanates or indoles such as thiocyanates, nitriles, cyano-epithioalkanes or oxazolidine-2-thiones might be formed depending on the reaction conditions.[4] Genetic variation in selected genes is also known to affect further metabolism. For example, isothiocyanates are conjugated with glutathione by glutathione *S*-transferases and subsequently converted to mercapturic acids, which can be excreted in urine.[37] Genetic polymorphisms in glutathione *S*-transferase genes leading to losses of activity of the encoded enzyme may result in slower metabolization and excretion of isothiocyanates, which in turn might extend the time of their biological action within the human body.[34]

Strengths of our study include the extensive and validated food frequency questionnaire used to assess habitual dietary intake,[28,29] the absence of specific recall bias for dietary data due to the prospective design and the high follow-up rates, which minimize the risk of selection bias. Additionally, we repeated our analyses after excluding the first 2 years of follow-up to exclude changes in diet due to not yet diagnosed cancer. The extensive data collection at recruitment enabled us to adjust for potential confounding factors. In comparison with the age-stratified model, multivariate adjustments strengthened the inverse association of GLS intake and risk of prostate cancer. Because consumption of GLS-containing vegetables increased with increasing vegetable intake, we adjusted our analyses additionally for the consumption of other vegetables (vegetables except GLS-containing vegetables) to rule out the possibility that the observed inverse associations might be due to other vegetable intake.

A major concern in studies on prostate cancer is the fact that PSA screening is getting more and more frequent. This may cause detection bias in populations not uniformly screened, because PSA testing increases the possibility of being diagnosed with prostate cancer at an early stage. Furthermore, people who participate in screening programs are supposed to have an overall healthier lifestyle, which might be positively associated with the exposure under study. As a consequence participation in PSA screening may act as confounder and mask a true association.[38] In our subanalysis restricted to participants who had at least 1 PSA test, we still found the same inverse association, which suggests that no substantial bias was introduced by selective participation in PSA screening.

In conclusion, our findings indicate an inverse association between GLS intake, especially aliphatic GLS, and risk of prostate cancer. This relationship was predominantly apparent in nonadvanced and low-grade cancer cases. Results based on the consumption of a most comprehensive group of GLS-containing foods, which can be seen as a proxy for GLS intake, gave similar although weaker results.

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